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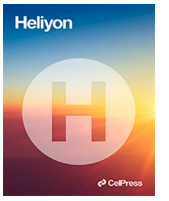

Research article

Bioinformatics-based analysis of the dialog between COVID-19 and RSA

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ABSTRACT

Pregnant women infected with SARS-CoV-2 in early pregnancy may face an increased risk of miscarriage due to immune imbalance at the maternal-fetal interface. However, the molecular mechanisms underlying the crosstalk between COVID-19 infection and recurrent spontaneous abortion (RSA) remain poorly understood. This study aimed to elucidate the transcriptomic molecular dialog between COVID-19 and RSA. Based on bioinformatics analysis, 307 common differentially expressed genes were found between COVID-19 (GSE171110) and RSA (GSE165004). Common DEGs were mainly enriched in ribosome-related and cell cycle-related signaling pathways.

Using degree algorithm, the top 10 hub genes (RPS27A, RPL5, RPS8, RPL4, RPS2, RPL30, RPL23A, RPL31, RPL26, RPL37A) were selected from the common DEGs based on their scores. The results of the qPCR were in general agreement with the results of the raw letter analysis. The top 10 candidate drugs were also selected based on P-values. In this study, we provide molecular markers, signaling pathways, and small molecule compounds that may associate COVID-19. These findings may increase the accurate diagnosis and treatment of COVID-19 patients.

1. Introduction

In 2019, a severe acute respiratory illness was reported caused by coronavirus disease 2019 (COVID-19) infection. Later in March 2020, it was declared a global pandemic [1]. The lungs are the most severely affected organs by SARS-CoV-2 infection, manifesting as diffuse alveolar damage, exudation, interstitial fibrosis, infiltration of immune cells, and expression of inflammatory cytokines [2–4]. Additionally, COVID-19 can also cause damage and inflammation in many other organs, resulting in multi-organ dysfunction and

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systemic inflammation [5]. In severe COVID-19 patients, the number of Th17 cells increases significantly, while the proportion of Treg cells decreases. The imbalance between regulatory T cells (Tregs) and Th17 cells leads to the uncontrolled release of pro-inflammatory cytokines (CS), which subsequently cause systemic inflammation and multi-organ failure, ultimately leading to death [6]. Pregnant women experience physiological changes in their cardiopulmonary and immune systems, making them more susceptible than usual to respiratory infections. These changes include diaphragmatic elevation, increased oxygen consumption, and respiratory mucosal edema, which make them less tolerant to hypoxia [7]. Previous studies have shown an increased risk of miscarriage in pregnant women infected with SARS-CoV-2 in early pregnancy, with potential mechanisms including increased inflammation, cytokine storm, hypercoagulability, and exposure to chronic stress [8].

During embryo implantation, a local inflammatory response occurs, which is crucial for the establishment of pregnancy [9,10]. However, successful maintenance of pregnancy depends on the timely resolution of the inflammatory response, followed by a transition of the maternal immune system to a tolerant state. Impaired immune tolerance due to uncontrolled inflammation or weakened immunosuppression can lead to immune-mediated complications of pregnancy, such as miscarriage, preterm birth, or fetal growth restriction [11]. The definition of recurrent spontaneous abortion (RSA) has not been uniformly defined internationally. The latest expert consensus in China defines RSA as the occurrence of two or more pregnancy losses before 28 weeks of gestation, including biochemical pregnancies [12]. The etiology of RSA is complex and mainly includes chromosomal or genetic abnormalities, anatomical abnormalities, autoimmune diseases, endocrine factors, infectious factors, male factors, and environmental psychological factors. There is also a subset of RSA with unknown causes, which are called unexplained recurrent spontaneous abortion (URSA) or immune-related RSA.

Most immune-related miscarriages are associated with impaired maternal immune tolerance as well as increased systemic and local pro-inflammatory cytokines and immune response activation [13–15]. In early pregnancy, a dynamic balance of pro-inflammatory and anti-inflammatory mediators is required for a normal pregnancy, such as Treg/Th17 and Th1/Th2. When the balance is disrupted and shifted towards a pro-inflammatory state, the maternal and fetus immune systems mutually reject each other, resulting in fetal loss [16,17]. Evidence indicates that in RSA, there is a disruption in the delicate equilibrium between pro-inflammatory and anti-inflammatory mediators in the uterine endometrium [18–20]. It has been reported that compared with women with a normal pregnancy, women with RSA have increased levels of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, interleukin (IL)-6, and IL-10 [21,22]. An aberrant cytokine uterine endometrial environment can impede embryo implantation and placental development through various mechanisms. An increase in pro-inflammatory cytokines, especially IFN-γ, can trigger macrophage activation, prompting their differentiation and expression of inducible nitric oxide synthase (iNOS) [23]. This can directly jeopardize the trophoblast layer. In addition, it has been observed that the proliferation and invasion of extravillous trophoblasts were inhibited by pro-inflammatory cytokines in vitro [24].

COVID-19 pneumonia is the result of widespread inflammation caused by viral replication in respiratory cells, which in severe cases triggers coagulation in the pulmonary vasculature, and the cytokines involved in this process are the same as those found in inflammation during pregnancy, suggesting that similar pathogenic mechanisms may exist between the two [25]. It has been reported that SARS-CoV-2 may affect reproductive health by inducing cytokine storms in infected pregnant women, leading to adverse pregnancy outcomes [26]. To further explore the interaction between COVID-19 and RSA, in this study, we searched the COVID-19 and RSA datasets to identify shared differentially expressed genes using the GEO public database. Subsequently, possible transcription factor binding sites (TFS), miRNAs, and related potential drugs were identified by functional enrichment and protein network interaction analysis. Finally, these core genes were validated by RT-qPCR experiments. It is hoped that these studies will provide some reference value for elucidating the pathogenesis of recurrent spontaneous abortion (RSA) infected with COVID-19.

2. Methods

2.1. Data acquisition

We obtained two datasets for our analysis: a COVID-19 dataset (GEO ID: GSE171110) consisting of 44 whole blood samples from COVID-19-infected individuals and 10 healthy whole blood samples, and a recurrent spontaneous abortion (RSA) dataset (GEO accession ID: GSE165004) consisting of 24 RSA and 24 healthy fertile control uterine endometrial samples. Both datasets were sequenced using Illumina HiSeq 2000 [27].

2.2. Identification of shared differentially expressed genes (DEGs) between COVID-19 and RSA

For the identification of genes with differential expression, we utilized the RStudio software (version 4.1.2) and employed the "Deseq2" package for the COVID-19 dataset (GSE171110), while the RSA dataset (GSE165004) was analyzed using the "limma" package. We selected differentially expressed genes (DEGs) from the COVID-19 dataset by applying the following criteria: $|\text{Log2 Fold Change}| > 0.585$ and $|\text{adj.P.Val}| < 0.05$. Similarly, for the RSA dataset, we identified DEGs with $|\text{Log2 Fold Change}| \geq 0.16$ and $|\text{adj.P.Val.}| < 0.05$. To identify the common DEGs between the COVID-19 and RSA datasets, we utilized the "Venn" package in R software.

2.3. GO and KEGG enrichment analysis of common differentially expressed genes

To explore the biological pathways associated with the shared DEGs, we performed gene ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using the "clusterProfiler" R package in R software. We considered functional terms

and pathways with a p-value <0.05 as the most significant, and they were listed for the common DEGs. P-value <0.05 was used to quantify the most significant functional terms and pathways listed for the common DEGs.

2.4. PPI network analysis based on common differentially expressed genes

Protein-protein interactions (PPIs) were analyzed by constructing a PPI network, which offers valuable insights into cellular mechanisms [28]. The common DEGs were submitted to the STRING website (https://string-db.org/) to assess protein-protein interactions. A comprehensive score greater than 0.6 was used to construct the PPI network for these common DEGs. The visualization of the protein-protein interaction (PPI) network was achieved through the utilization of Cytoscape software (version 3.9.1).

2.5. Extraction of hub genes

The CytoHubba plugin in Cytoscape 3.9.1 was used to identify the top 10 hub genes by ranking them according to the degree algorithm [29]. Cytoscape is a valuable tool for evaluating and determining biological network regulators using network metrics [30]. We conducted receiver operating characteristic (ROC) analysis to assess the diagnostic value of the top 10 hub genes in COVID-19 and RSA.

2.6. Real-time PCR was used to verify the hub genes

Total RNA from decidual tissue was extracted from RSA (n = 5) and normal fertile controls (n = 5), and then reverse transcribed into cDNA (RR047A, Takara, Japan). The study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (2023-E365-01). A real-time PCR system was used with Takara SYBR Premix Ex *Taq*II (Tli RNaseH Plus) (RR820A, Takara, Japan). Reactions were run under the following conditions: 30 s at 95 °C, followed by 40 cycles of 5 s at 95 °C and 30 s at 60 °C, and then 95 °C for 15 s, 60 °C for 1min. Finally, 15 s at 60 °C. GAPDH was chosen as the internal reference gene, and the relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method (see Table 1 for primers used) and normalized with respective controls.

2.7. Identification of transcription factors and miRNAs regulating hub genes

Transcription factors (TFs) regulate protein expression by binding to specific DNA sequences [31]. Enrichr datebase (https://maayanlab.cloud/Enrichr/) is an online enrichment analysis tool that offers various visual summaries for gene lists [32]. To identify common transcription factors in the differentially expressed genes (DEGs), we employed Transcription Factor PPIs tool in Enrichr to identify TFs and constructed interaction maps using Cytoscape software (version 3.9.1). Additionally, we investigated target gene-miRNA interactions to identify miRNAs that may negatively regulate protein expression by destabilizing mature messenger RNAs and reducing translation efficiency [33]. For experimentally validated miRNA-target interactions, we sourced information from miRTarBase [34]. Next, we utilized the miRTarBase 2017 library analysis tool in Enrichr to identify miRNAs associated with the common DEGs. To visualize the interaction between miRNAs and genes, we employed Cytoscape software (version 3.9.1) to create

Table 1
Primers used in this study.

Primer Name	Prime (5′ to 3′)	Length (bp)
RPS27A-F	TTGAGACTTCGTGGTGGTGC	129
RPS27A-R	TTTGCCATTCTCATCCACCTTA	
RPL5-F	GGTCTCTGTTCCGCAGGATG	128
RPL5-R	CACCAAGCGTTTCCGAGCAT	
RPS8-F	TTTGCGGTTTCTCTTTCCAGC	194
RPS8-R	TTCTTGTTACCTCCCCGCAC	
RPL4-F	GGGCATGTGGGACGTTTCTG	199
RPL4-R	GGATCTTCTTGCGTGGTGCT	
RPS2-F	GGATAAGGAGTGGATGCCCGT	137
RPS2-R	GAGAGGCCCCCAGGAAGAAAT	
RPL30-F	CTCGTTCCCCGGCCATCTTA	103
RPL30-R	GACTCCAGCGACTTTTTCGTC	
RPL23A-F	GCCAAGGTCAACACCCTGAT	198
RPL23A-R	CCCAGCCCAACCAGAAATTG	
RPL31-F	GGGCCAAAGGAATAAGGAATGTG	146
RPL31-R	TGGGATGGAGAACTTACTTTTGA	
RPL26-F	TACAACGTGCGATCCATGCC	153
RPL26-R	GCCATTAGCCTTTTCCCGCT	
RPL37A-F	AGAAAGTCGGGATCGTCGGT	146
RPL37A-R	GATCCCCACAGCTCGTCTCT	
GAPDH-F	CACCGTCAAGGCTGAGAACG	141
GAPDH-R	ATGGTGGTGAAGACGCCAGT	

miRNA gene interaction maps.

2.8. Evaluation of candidate drugs

To identify potential drugs for COVID-19 treatment, we compared the common DEGs with Drug Signatures Database (DSigDB) analysis tool in Enrichr (https://maayanlab.cloud/Enrichr/) and selected the top 10 candidate drugs with the lowest p-values for further analysis.

2.9. Gene-disease association analysis of hub genes

We used DisGeNET database (https://www.disgenet.org/) to identify the top ten drugs with P-values, Cytoscape software (version 3.9.1) to create gene-*disease* interaction maps. The DisGeNET is a versatile platform that serves various research purposes. It can be utilized to explore the molecular underpinnings of specific human diseases and their comorbidities, classify disease genes, formulate hypotheses regarding the therapeutic effects and side effects of drugs, and much more [35–38].

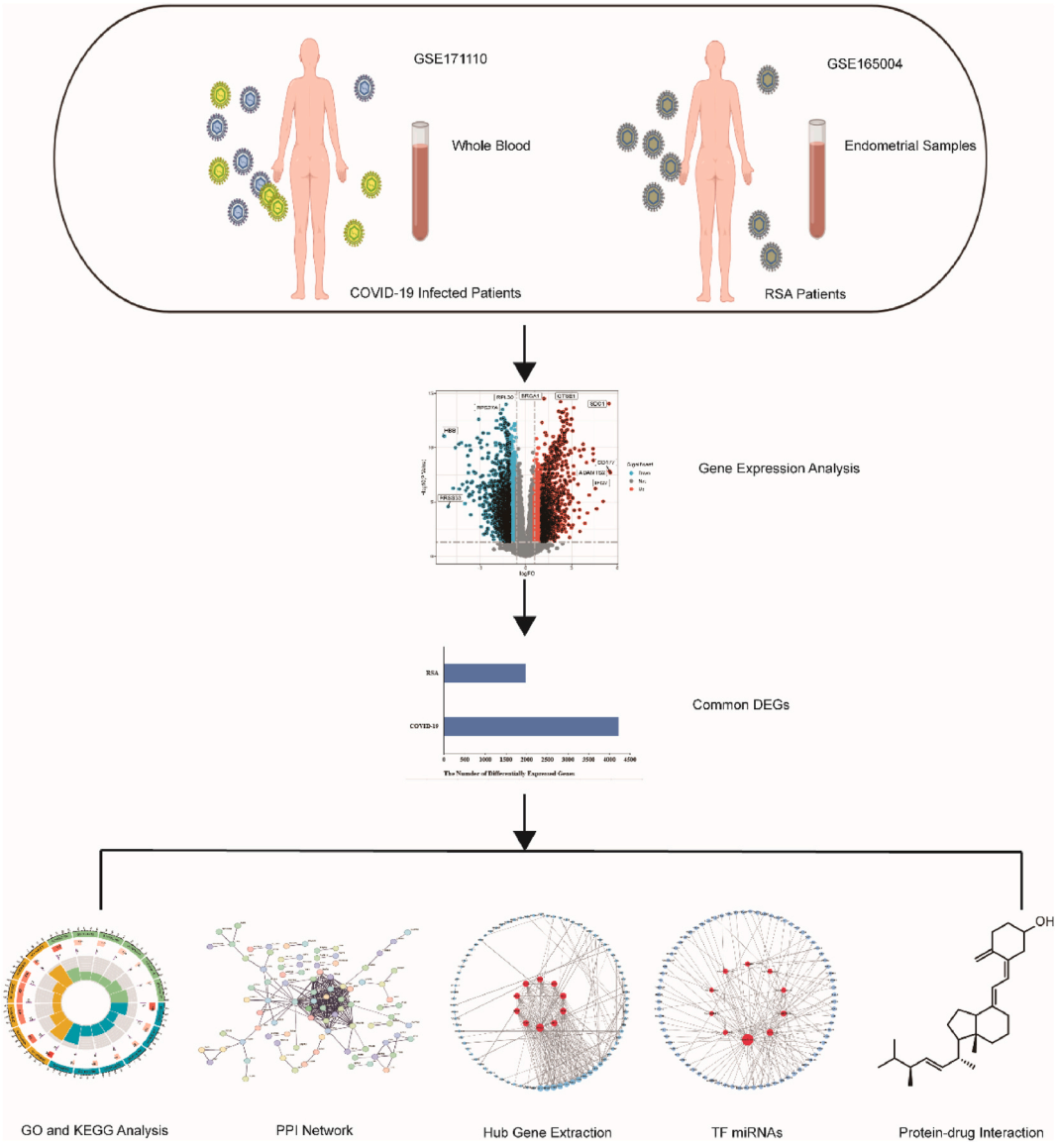


Fig. 1. Above is the comprehensive workflow diagram illustrating the steps of our research.

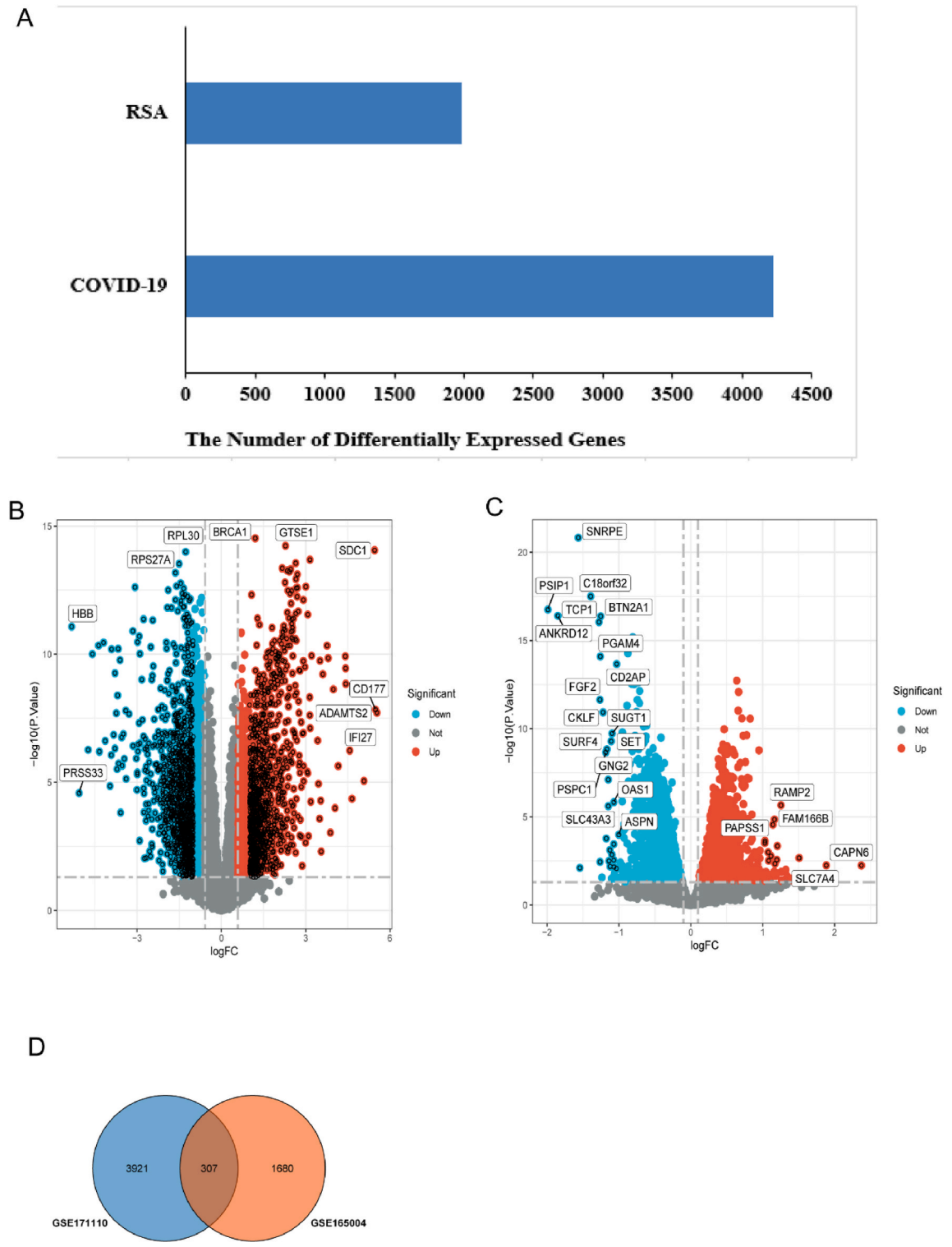


Fig. 2. The visualization illustrates the count of shared differentially expressed genes in two datasets, COVID-19 (GSE171110) and RSA (GSE165004). (A) A comparison of the number of differentially expressed genes was performed between the COVID-19 and RSA datasets. (B) The graph illustrating the differential expression of genes in COVID-19 datasets using a volcano plot. (C) A volcano plot was created to illustrate the differentially expressed genes in the RSA dataset. (D) A Venn diagram was constructed to demonstrate the overlap of differentially expressed genes between the COVID-19 and RSA datasets.

3. Results

3.1. Identification of common DEGs between COVID-19 and RSA

We have represented all the crucial processes of our study in a flowchart (Fig. 1). To investigate the interaction between COVID-19 and RSA, blood samples from the GEO database were analyzed in this study. Fig.S4-S5 present boxplots of gene expression data for COVID-19 and RSA datasets, both before and after normalization. In the COVID-19 dataset, a total of 4228 DEGs were identified, meeting the criteria of |Log2 Fold Change| > 0.585 and |adj.P.Val|<0.05 (Fig. 2A). Similarly, in the RSA dataset, 1987 DEGs were identified with |Log2 Fold Change| ≥0.16 and |adj.P.Val.|<0.05 (Fig. 2A). Moreover, volcano plots were generated individually for COVID-19 and RSA DEGs (Fig. 2B and C; tables S3 and S4). A Venn diagram depicts that 307 DEGs are shared between the COVID-19

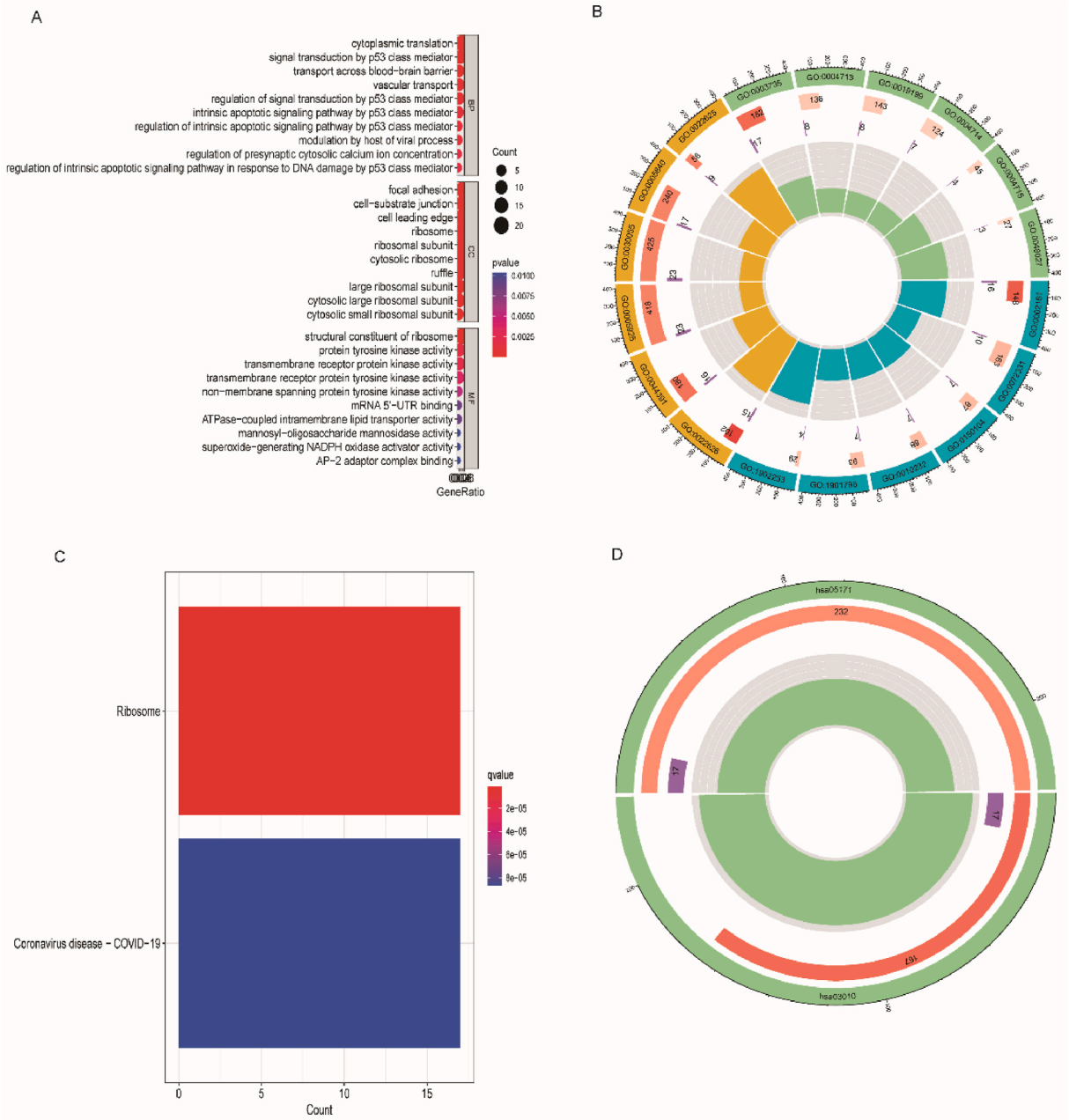


Fig. 3. Functional enrichment analysis was performed for the common differentially expressed genes. (A) Bubble graphs were generated to display the results of the GO enrichment analysis. (B) A circle diagram was created to visualize the GO enrichment analysis. (C) Bar graphs were used to present the findings of the KEGG enrichment analysis. (D) A circle diagram was constructed to illustrate the results of the KEGG enrichment analysis.

and RSA datasets (Fig. 2D). These findings demonstrate a significant overlap in gene expression, implying a close relationship between RSA and COVID-19.

3.2. GO and KEGG enrichment analysis of common DEGs between COVID-19 and RSA

GO and KEGG enrichment analyses are commonly employed to reveal the interconnections between genes and their associated terms, as well as pathways [39]. To identify enriched biological features and pathways among the commonly differentially expressed genes (DEGs), we employed the "ClusterProfiler" package. GO enrichment analysis was performed to identify significant enrichment pathways (p-value <0.05) in terms of biological processes (BP), cellular components (CC), and molecular functions (MF). Significant pathways in the BP category included cytoplasmic translation, signal transduction by p53 class mediator, and transport across blood-brain barrier. In the CC category, focal adhesion, cell-substrate junction, and cell leading edge emerged as the top three. Additionally, the top three statistically significant items in the MF aspect were structural constituents of ribosome, protein tyrosine kinase activity, and transmembrane receptor protein kinase activity (Fig. 3A and B). KEGG enrichment analysis identified two pathways, ribosome, and coronavirus disease - COVID-19 (Fig. 3C and D). These findings imply a robust correlation between these common DEGs and ribosome, which could potentially pave the way for more effective treatments for COVID-19 and RSA.

3.3. Construction of PPI network and identification of hub genes

We used STRING to analyze protein-protein interactions among the common DEGs in COVID-19 and RSA. Fig. 4 illustrates the interactions among commonly differentially expressed genes (DEGs) in both RSA and COVID-19. The top 10 hub genes were ranked based on their scores, which were determined by their degree in the protein-protein interaction (PPI) network using Cytoscape, and they were RPS27A, RPL5, RPS8, RPL4, RPS2, RPL30, RPL23A, RPL31, RPL26, and RPL37A (Fig. 5). Subsequently, ROC analysis was independently conducted for RSA and COVID-19 datasets. In the RSA dataset, all hub genes exhibited AUC values exceeding 0.616, whereas in the COVID-19 dataset, all hub genes demonstrated AUC values surpassing 0.973 (Fig.S2, S3). These findings suggest the

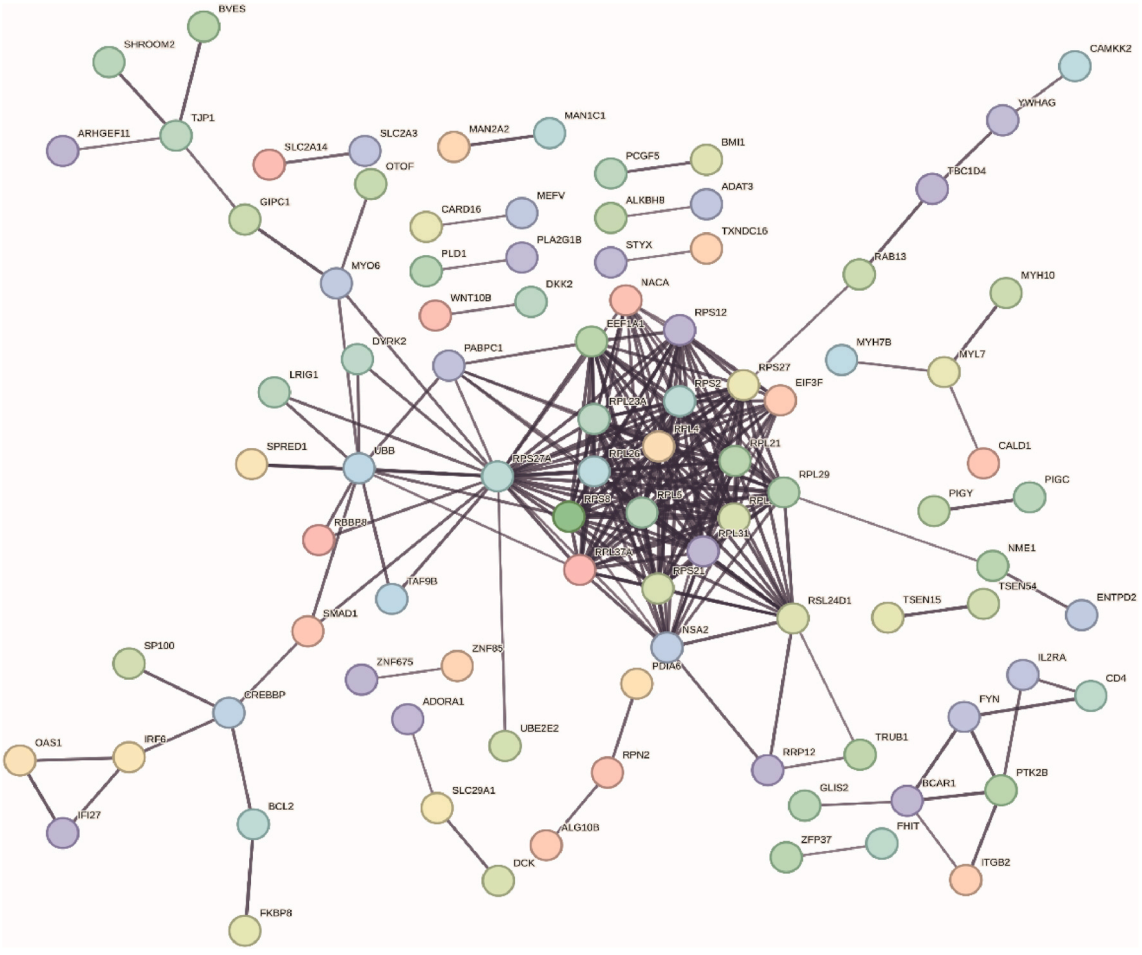


Fig. 4. PPI-network analysis reveals common differentially expressed genes between COVID-19 and RSA.

possibility of developing new targeted therapies for COVID-19 by focusing on these hub genes.

3.4. Experimental validation of hub gene expression

To validate the expression of signature genes associated with RSA, we collected 5 healthy fertile controls and 5 recurrent spontaneous abortion decidual tissues for clinical sample validation. The results demonstrate that the mRNA expression levels of RPS27A and RPL5 in the RSA group were higher than those in the control group, and these differences were statistically significant (Fig. 6A–B). Although the mRNA expression levels of RPS8, RPL4, RPS2, RPL30, RPL23A, and RPL31 did not differ significantly between the two groups, there was a trend of higher mRNA expression levels in the RSA group compared to the control group (Fig. 6C–H). The mRNA expression level of RPL26 was significantly higher in the RSA group compared to the control group ($P = 0.03$, Fig. 6I). Additionally, the expression level of RPL37A was significantly lower in the control group compared to the RSA group ($P < 0.05$, Fig. 6J). The expression pattern of these 10 hub genes in the GSE165004 dataset was consistent with that of the clinical specimens, further suggesting their potential diagnostic value in predicting RSA progression (Fig. 6).

3.5. Construction of regulatory networks

Gene expression regulatory factors can be divided into two types: transcription factors (TFs) and miRNAs. TFs regulate

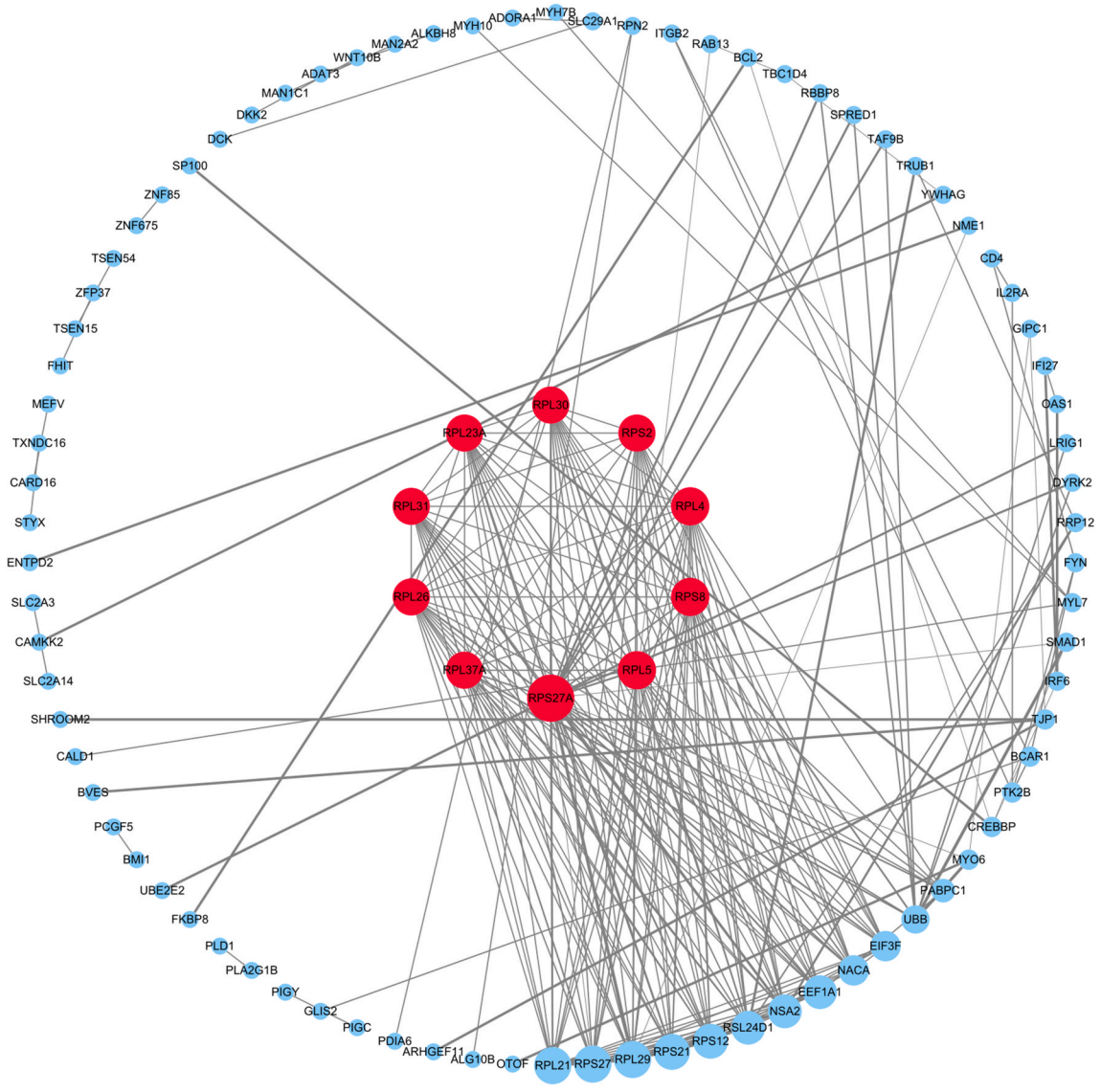


Fig. 5. The figure shows the top 10 hub genes.

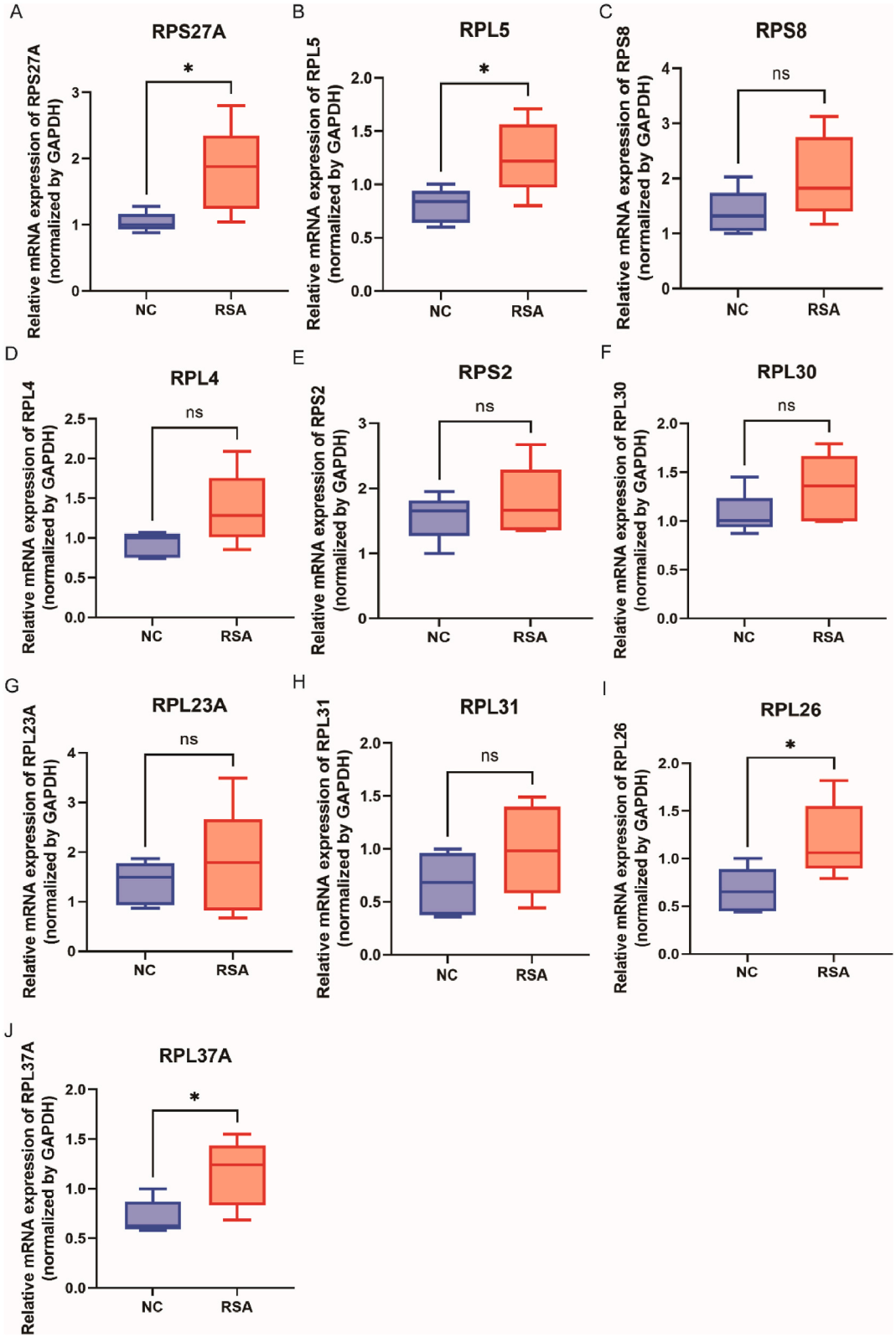


Fig. 6. Expression validation of hub genes between control and RSA groups. (A) RPS27A, (B) RPL5, (C) RPS8, (D) RPL4, (E) RPS2, (F) RPL30, (G) RPL23A, (H) RPL31, (I) RPL26, (J) RPL37A, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

transcription by binding to promoter regions, whereas miRNAs modulate gene expression post-transcription [40]. The analysis of interactions between TFs and miRNAs showed that 165 TFs and 2466 miRNAs coordinated these common DEGs, indicating a close collaboration between them. Fig. 7 shows the top 10 ranked TFs based on their p-values, which were ILF3, RAD21, ESR1, HOXD13, ILF2, LMO4, NFE2L2, TCF3, CRX, and CDX2. The top 10 miRNAs were also ranked based on their p-values, including hsa-miR-16-5p, hsa-miR-8069, hsa-miR-154-5p, hsa-miR-100-5p, hsa-miR-103a-3p, hsa-miR-365a-3p, hsa-miR-892c-5p, hsa-miR-4801, hsa-miR-4731-3p, and hsa-miR-106b-3p (Fig. 8). These results indicate a strong correlation between common DEGs and TFs, as well as miRNAs.

3.6. Identification of candidate drugs

Fig. 9 shows the top 10 ranked drugs (METHYL METHANESULFONATE CTD 00006307, verteporfin HL60 DOWN, vincristine CTD 00006988, cicloheximide HL60 DOWN, VALPROIC ACID CTD 00006977, tetrahydropalmatine CTD 00000745, etifenin PC3 DOWN, rifabutin PC3 UP, FITC BOSS, and flunisolide HL60 UP). These small molecular compounds have the potential to be used as therapeutic targets for the treatment of COVID-19 and RSA.

3.7. Disease association identification

It has been recognized that there is an interrelationship between various diseases, indicating the presence of at least one or more common genes [41]. We utilized the DisGeNET database (https://www.disgenet.org/) to identify 10 diseases that exhibited significant associations with commonly differentially expressed genes (DEGs), such as Aase Smith syndrome 2, Aase Smith syndrome 2, Arterial Occlusive Diseases, Carcinoma Large Cell, Histiocytic Necrotizing Lymphadenitis, Infant Acute Lymphoblastic Leukemia, Lymphoma, Follicular, Pontocerebellar Hypoplasia Type 2, Pseudolymphoma, Self-Mutilation, and Smooth Muscle Tumor (Fig.S1). These results suggest commonalities between these diseases and RSA as well as COVID-19.

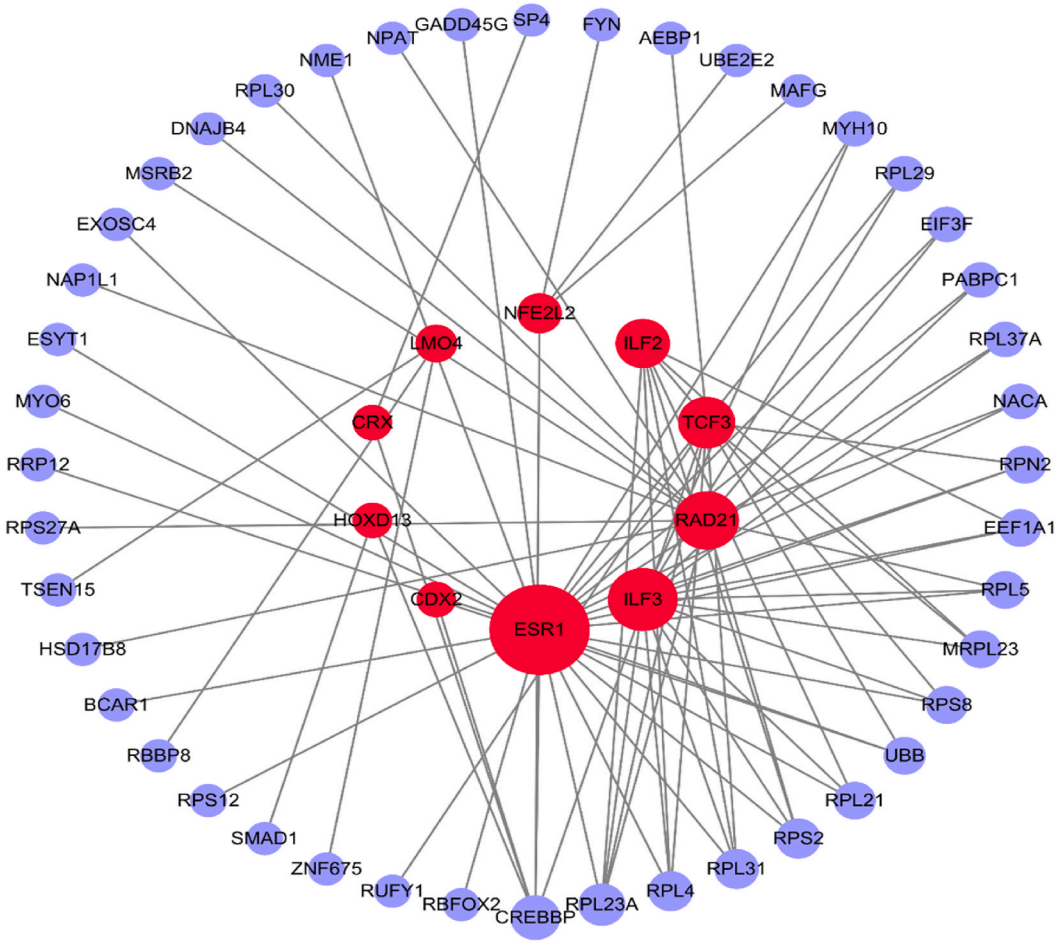


Fig. 7. The top 10 TFs were ranked based on their P values and their interactions with common differentially expressed genes.

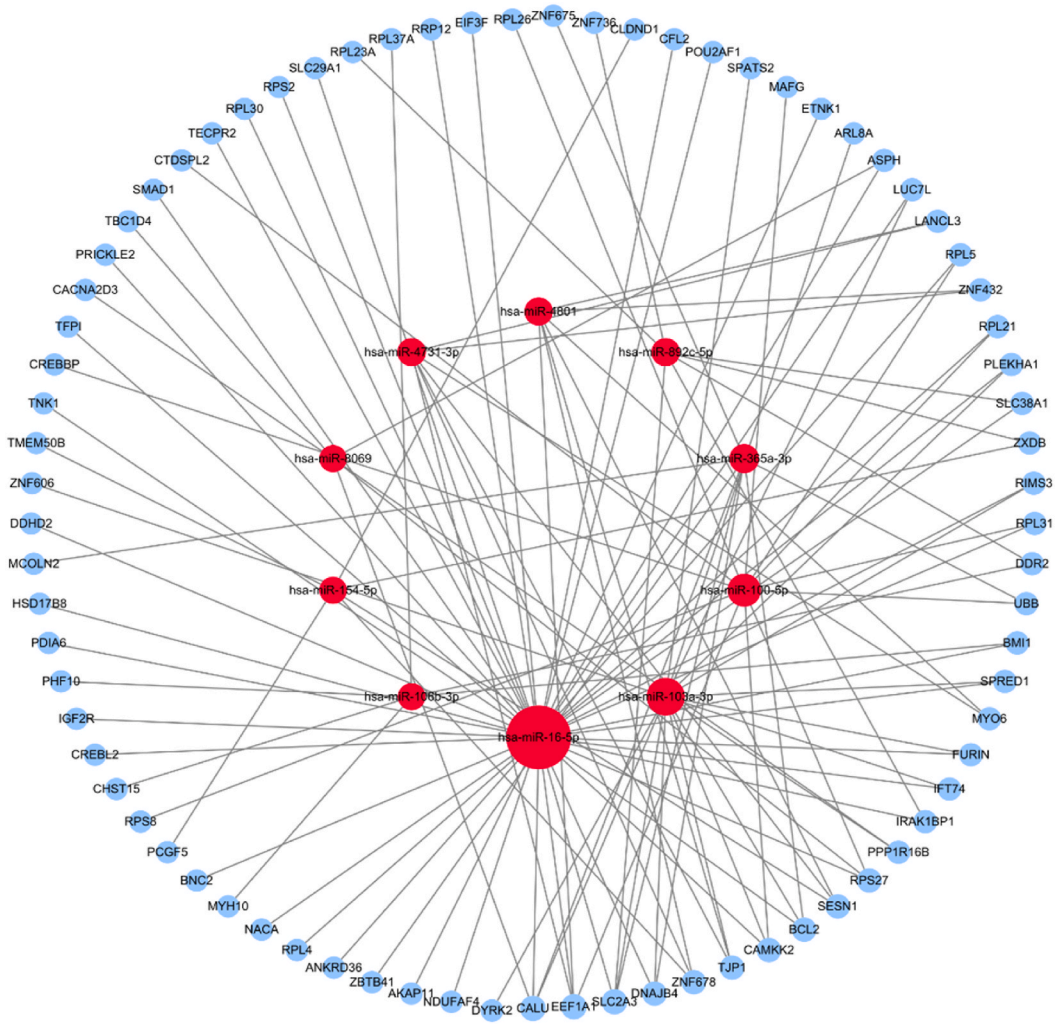


Fig. 8. The top ten miRNAs were ranked according to the most significant difference in p-values.

4. Discussion

In recent years, many studies have shown the potential association between different diseases. Consequently, exploring the interaction between various diseases has emerged as a promising field that requires further investigation in the future [42–44]. COVID-19 has caused a significant number of deaths worldwide, posing major challenges to public health. RSA affects 2–5% of couples of reproductive age [45], and its underlying pathophysiological mechanism remains unclear. Some known risk factors associated with RSA include advanced maternal age, chromosomal abnormalities, immune factors, maternal anatomical abnormalities, and maternal comorbidities such as thrombotic disorders and infections [46]. Women with a history of RSA have a lower rate of successful pregnancies, which not only severely affects the reproductive health of women but also brings immense physical and mental distress to patients and their families. The etiology of RSA is partially unknown and is linked to immune imbalances at the interface between the mother and fetus, which include changes in immune cytokines. The association between COVID-19 infection and the likelihood of RSA remains a subject of ongoing debate. In this study, we analyzed public databases on COVID-19 and RSA to discover potential connections between the two diseases. We obtained the COVID-19 dataset (GSE171110) and the RSA dataset (GSE165004) from the GEO database. Differential analysis was then performed on these datasets. The overlapping DEGs generated from these two sets were further analyzed, and a degree-based ranking was used to identify 10 hub genes (RPS27A, RPL5, RPS8, RPL4, RPS2, RPL30, RPL23A, RPL31, RPL26 and RPL37A). Central proteins can be used for the development of therapeutic interventions.

Ribosomal proteins are named RPL (ribosomal protein large) and RPS (ribosomal protein small) according to their origin from the large and small subunits of ribosomes, respectively. Current research has proven that ribosomal protein families have a broader impact by overseeing the expression of oncogenes and tumor suppressor genes, controlling the cell cycle and apoptosis, promoting angiogenesis, coordinating chromosomal genes, and regulating tumor proliferation, invasion, and metastasis. Several members of the ribosomal protein family inhibit tumor growth through the MDM2/MDMX-p53 pathway. RPS27A is a potential biomarker for sperm

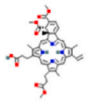
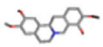
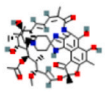
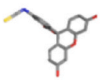
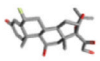
Name	P-value	Chemical Formula	Structure
METHYL METHANESULFONATE CTD 00006307	7.63E-05	$C_2H_6O_3S$	
verteporfin HL60 DOWN	1.30E-04	$C_{82}H_{84}N_8O_{16}$	
vincristine CTD 00006988	1.94E-04	$C_{46}H_{56}N_4O_{10}$	
cicloheximide HL60 DOWN	3.91E-04	$C_{15}H_{23}NO_4$	
VALPROIC ACID CTD 00006977	4.03E-04	$C_8H_{16}O_2$	
tetrahydropalmatine CTD 00000745	5.03E-04	$C_{21}H_{25}NO_4$	
etifenin PC3 DOWN	5.04E-04	$C_{16}H_{22}N_2O_5$	
rifabutin PC3 UP	5.54E-04	$C_{46}H_{62}N_4O_{11}$	
FITC BOSS	5.66E-04	$C_{21}H_{11}NO_5S$	
flunisolide HL60 UP	6.23E-04	$C_{24}H_{31}FO_6$	

Fig. 9. Ten potentially effective drugs were screened.

vitality, and low expression of RPS27A in sperm may lead to lower pregnancy rates after fertilization [47,48]. It is also involved in ribosome biogenesis and ubiquitin production and plays an important role in cancer development. RPS27A is both an activator and a target molecule of p53 [49]. RPL5 upregulates p53 by binding and inhibiting MDM2 ubiquitin ligase, thereby inhibiting tumorigenesis [50]. Ribosomal protein S8 (RPS8) acts in synergy with cyclin-dependent kinase member CDK11p46 to inhibit protein translation in a cap-dependent and internal ribosome entry site (IRES)-dependent manner, sensitizing cells to apoptosis induced by Fas ligand [51]. The N-terminal of PRL4 is crucial for the establishment of Epstein-Barr virus (EBV)-associated persistent B lymphocyte infection related to tumor development [52]. RPS2 interacts with MDM2 through its RING finger domain, inhibiting MDM2 and inducing p53 and its downstream pathways [53]. RPL30 plays an important role in neural formation and Neural tube defects (NTDs) risk [54]. RPL23A regulates the protein expression of c-Myc and p21 [55]. Knockdown of the RPL31 gene may lead to nucleolar protein-mediated MDM2 inhibition, p53 stabilization and activation, and MDM2 accumulation, making MDM2 functionally ineffective in p53 degradation [56]. RPL26 can not only bind to MDM2 but also promote p53 mRNA translation [57]. RPL37 directly interacts with MDM2 [58].

As earlier studies have suggested, the imbalance of immune cytokines in RSA patients may be associated with an increased risk of SARS-CoV-2 positivity [25]. These findings underscore the pressing requirement for mechanistic research to gain a deeper understanding of how immune dysfunction associated with RSA influences the acquisition and elimination of infections. In this study, we performed GO and KEGG analyses to examine the correlation between COVID-19 and RSA. The “clusterProfiler” package was used to

perform GO analysis on three types of BPs, CCs, and MFs. The BPs of these common DEGs were mainly enriched in cytoplasmic translation, p53-like mediator signaling, and transport across the blood-brain barrier. p53, as a transcription factor, activates and inhibits an increasing number of target genes, involving cell cycle control, apoptosis, programmed necrosis, autophagy, metabolism, stem cell homeostasis, angiogenesis, and aging [59]. It is noteworthy that decreased levels of p53 are associated with severe respiratory diseases, and p53 protects against lung injury by antagonizing NF-kB-mediated inflammation/immune responses, indicating a protective role for p53 in vascular homeostasis and lung inflammation [60]. After SARS-CoV-2 infection, important molecules such as ACE2, the virus-host cell entry mediator p53, and the transcriptional regulatory factor NF-kB, which are involved in the RAS pathway, undergo changes, which are considered to be the main reasons for impaired immune responses and excessive cytokine release [61].

Additionally, we investigated the correlation among transcription factors (TFs), microRNAs (miRNAs), and common DEGs. miRNAs are short non-coding RNA that regulate the expression of target genes by binding to specific sites on mRNA [40]. miRNAs play a crucial role in numerous biological processes by controlling target genes, some of which have the potential to either facilitate cancer development or impede tumor growth [62]. Transcription factors (TFs) are proteins that interact with particular DNA sequences in order to control the process of transcription and the expression of genes [63]. TFs have crucial functions in numerous biological processes, as they bind to particular gene sequences, regulate gene transcription, govern metabolism, and impact immunity [64]. The top 10 TFs ranked by P-value were ILF3, RAD21, ESR1, HOXD13, ILF2, LMO4, NFE2L2, TCF3, CRX, and CDX2. The top 10 miRNAs sorted by p-value are hsa-miR-16-5p, hsa-miR-8069, hsa-miR-154-5p, hsa-miR-100-5p, hsa-miR-103a-3p, hsa-miR-365a-3p, hsa-miR-892c-5p, hsa-miR-4801, hsa-miR-4731-3p, hsa-miR-106b-3p. ILF3 and ILF2 promote HIV infection through direct interaction with vRNA [65]. Estrogen receptor (ER) signaling is crucial for a successful pregnancy. ER1 gene polymorphism is associated with URSA in the Chinese Han population [66]. A large clinical case-control study also showed that specific ESR1 variants increase the risk of RSA [67]. Estrogens interact with ESR1/2 receptors to protect COVID-19 patients by inhibiting inflammation and immune responses caused by SARS-CoV-2 infection [68]. Upregulation of LMO4 inhibits hematopoietic stem/progenitor cell generation in COVID-19 patients [69]. NFE2L2, a key transcription factor, controls the antioxidant enzymes as part of the cellular protective response to oxidative stress, in which NFE2L2 is the main transcription factor [70]. Increased levels of hypoxia-inducible factor 1-alpha (HIF1A) in decidua promote RPL through the TCF3/p38 signaling pathway [71]. VDR gene variations, such as the polymorphic locus CDX2, are significantly associated with the severity of COVID-19 and clinical outcomes in COVID-19 patients [72]. hsa-miR-16-5p may affect SARS-CoV-2 infection by regulating the ACE2 receptor-associated network [73]. The effects of other transcription factors and miRNAs on COVID-19 and RSA need further investigation.

Furthermore, we conducted gene-disease analysis to ascertain prevalent DEGs linked to disorders. The results showed that common DEGs in RSA and COVID-19 are associated with various diseases, including Ondine's curse, arterial occlusive diseases, large cell carcinoma, histiocytic necrotizing lymphadenitis, infant acute lymphoblastic leukemia, follicular lymphoma, pontocerebellar hypoplasia type 2, pseudo lymphoma, self-mutilation, and leiomyoma. Severe acute respiratory syndrome coronavirus 2 attaches to the angiotensin converting enzyme (ACE) 2 receptor and enters cells, leading to complications in the cardiovascular system, where ACE2 receptors are widely distributed [74]. Excessive cytokine production in COVID-19 leads to arterial obstruction [75]. Malignant hematologic disease patients have an increased risk of COVID-19 infection and tend to have more severe disease and higher mortality rate [76]. Our findings align with this outcome.

In this study, we identified various compounds and drugs that might be potential treatments for COVID-19 and RSA, including methamphetamine, vitexin, vincristine, hexamethylenediamine, valproic acid, Pueraria, etomidate, levofloxacin, isothiocyanate fluorescein, and flunisolide. However, there is currently no literature supporting the therapeutic effects of these drugs in COVID-19 and RSA. Developing a safe and effective drug treatment for COVID-19 patients with RSA remains an urgent priority.

The incidence of miscarriage is elevated in pregnant women who contract SARS-CoV-2 during the early stages of pregnancy [8]. Identifying the risk of infection in RSA patients early and intervening promptly is a key focus. However, no molecular blood biomarkers specifically for RSA patients infected with COVID-19 have been reported. In this study, we conducted ROC analysis on the top 10 hub genes (RPS27A, RPL5, RPS8, RPL4, RPS2, RPL30, RPL23A, RPL31, RPL26 and RPL37A) sorted by score. Our analysis revealed that all of these key genes had AUC values above 0.729 in the RSA cohort and AUC values above 0.950 in the COVID-19 cohort. The identification of these molecular blood biomarkers may offer novel insights for the diagnosis, care, and treatment of RSA patients infected with COVID-19. It can be seen that the expression levels of RPS27A, RPL5, RPL26, and RPL37A in RSA are consistent with the results of bioinformatics analysis. In the future, we can focus on these genes to explore the specific biological functions of these 4 core genes. As COVID-19 is now well controlled and the source of samples is difficult, we will conduct further verification in RSA samples, and also verify the relationship between the four core genes and pregnancy outcome through the aborted mouse model. We look forward to deeper basic research to clarify the interaction mechanism between these core genes and RSA.

In conclusion, our study has several strengths. First, we identified hub genes that have a significant impact on the onset and progression of COVID-19 and RSA by analyzing blood samples obtained from the GEO database. Second, we elucidated the interaction between COVID-19 and RSA, providing new insights into the molecular mechanisms of SARS-CoV-2 and RSA. Third, we identified 10 candidate drugs based on p-value sorting, which could potentially function as biomarkers for treating patients with COVID-19 and RSA.

Nonetheless, there are some limitations to this study. As the data mining was conducted using the GEO public database, further basic experiments are necessary to explore and validate the specific biological functions of the 10 selected hub genes. Furthermore, the dosage of these drug candidates needs to be evaluated in animal studies before progressing to clinical use. Additionally, a deeper exploration of the molecular mechanisms underlying SARS-CoV-2 and RSA is required.

5. Conclusion

In our study, we have identified potential molecular targets, signaling pathways, small molecules, and promising biomarkers that could potentially contribute to adverse pregnancy outcomes in RSA patients infected with COVID-19. These findings may aid in the precise diagnosis and treatment of RSA patients who have contracted SARS-CoV-2 infection.

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Ethics approval and consent to participate

This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University on August 1, 2023, approval number 2023-E365-01.

Data availability statement

The COVID-19 and RSA data used in this manuscript are available in the public database GEO (https://www.ncbi.nlm.nih.gov/geo/). The ID numbers are GSE171110 and GSE165004.

CRediT authorship contribution statement

Yin Bi: Writing – review & editing, Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Ting Li:** Writing – review & editing, Validation, Project administration, Methodology, Investigation, Data curation. **Shun Zhang:** Writing – review & editing, Funding acquisition, Formal analysis. **Yihua Yang:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Mingyou Dong:** Writing – review & editing, Visualization, Supervision, Software, Project administration, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30371.

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